Dual Targeting of EGFR and of PAM pathway: An innovative Peptide-Based therapeutic strategy for anaplastic thyroid carcinoma

Zehra-Cagla Kahvecioglu[1], Rachel Baillif[1], Samuel Vandecasteele[1], Marine Bougard[1], Alison Daubry[1], Olivia Rasson[1], Céline Martens[1], Maurine Appels[1], Sophie Laurent[1,2], Sven Saussez[3], Carmen Burtea[1]

[1]*NMR and Molecular Imaging Laboratory, General, Organic and Biomedical Chemistry Unit, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons, B-7000 Mons, Belgium*
[2]*Center for Microscopy and Molecular Imaging (CMMI), Charleroi, Belgium*
[3]*Human Anatomy and Experimental Oncology Unit, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons, B-7000 Mons, Belgium*

zehracagla.kahvecioglu@umons.ac.be

Background
Despite its rarity, anaplastic thyroid carcinoma (ATC) ranks among the most aggressive and lethal endocrine malignancies, marked by rapid progression, resistance to conventional therapies, and a median overall survival of less than six months following diagnosis [1]. Although it represents only 1–2% of all thyroid malignancies, ATC accounts for up to 39% of thyroid cancer–related deaths [2]. This high rate of mortality reflects its aggressive biological behavior and the limited efficacy of current treatment modalities. The urgent need for novel therapeutic strategies is driven by ATC's resistance to conventional therapies and its reliance on dysregulated intracellular signaling pathways. Among the most critical molecular features of ATC is the overactivation of the PI3K/AKT/mTOR (PAM) pathway, a central regulator of tumor cell survival, proliferation, and resistance to apoptosis [3]. In parallel, overexpression of the epidermal growth factor receptor (EGFR) has been consistently associated with increased tumor invasiveness and poor clinical outcomes [4]. Together, the dysregulated PAM signaling pathway and EGFR overexpression represent major contributors to ATC aggressiveness [3,4].

Methods
We engineered a dual-function peptide complex (PC) that combines a vector peptide (VP), targeting EGFR overexpression, with a therapeutic peptide (TP), designed to disrupt PIP3-mediated protein interactions, thereby impairing AKT activation. Both peptides were selected using phage display technology and chemically conjugated. EGFR and AKT expression were evaluated in ATC cell lines (8505c, Cal-62) and normal thyroid cells (Nthy-ori 3-1) using Western Blot, immunofluorescence (IF), and immunohistochemistry (IHC). Apoptotic activity was quantified by Annexin V flow cytometry and cleaved caspase-3 detection. Subcellular trafficking was assessed via IF co-localization of EGFR–endoplasmic reticulum (ER), VP–ER, and VP–caveolae. *In vivo* safety of VP was assessed in NMRI mice, while therapeutic efficacy of PC was tested in athymic nude mice bearing Cal-62 or 8505c tumors. Biodistribution was evaluated by optical imaging using IRDye800CW-conjugated VP.

Results
ATC cells showed elevated EGFR and AKT activation, with nuclear localization of p-AKT (S473). PC induced significantly greater apoptosis than TP alone, with an EC_{50} of 5 µM (vs. IC_{50} of 24 µM for TP), with strong caspase-3 activation and pyknotic nuclear changes confirmed by Masson's Trichrome staining. IF analyses revealed retrograde trafficking of VP and nuclear AKT accumulation. *In vivo*, PC administration led to marked tumor regression without histological evidence of systemic organ toxicity. Minor hepatic stress and elevated BUN were observed, possibly linked to anesthesia. CYP450 profiling indicated moderate inhibition of CYP3A4 by P2 and PC. Fluorescence imaging confirmed tumor-specific accumulation of VP, validating its targeting efficacy.

Conclusion
This novel peptide-based approach enables dual targeting of EGFR and PIP3 to selectively inhibit PAM pathway in ATC cells. The peptide complex demonstrates potent antitumor activity, efficient intracellular delivery, and minimal systemic toxicity, supporting its translational potential as a targeted therapy for aggressive thyroid malignancies.

References:

1) Molinaro E, Romei C, Biagini A, Sabini E, Agate L, Mazzeo S, Materazzi G, Sellari-Franceschini S, Ribechini A, Torregrossa L, Basolo F, Vitti P, Elisei R. Anaplastic thyroid carcinoma: from clinicopathology to genetics and advanced therapies. *Nat Rev Endocrinol.* 2017 Nov;13(11):644-660. doi: 10.1038/nrendo.2017.76. Epub 2017 Jul 14. PMID: 28707679.
2) Smallridge RC, Copland JA. Anaplastic thyroid carcinoma: pathogenesis and emerging therapies. *Clin Oncol* (R Coll Radiol). 2010 Aug;22(6):486-97. doi: 10.1016/j.clon.2010.03.013. Epub 2010 Apr 24. PMID: 20418080; PMCID: PMC3905320.
3) Pozdeyev N, Gay LM, Sokol ES, Hartmaier R, Deaver KE, Davis S, French JD, Borre PV, LaBarbera DV, Tan AC, Schweppe RE, Fishbein L, Ross JS, Haugen BR, Bowles DW. Genetic Analysis of 779 Advanced Differentiated and Anaplastic Thyroid Cancers. *Clin Cancer Res.* 2018 Jul 1;24(13):3059-3068. doi: 10.1158/1078-0432.CCR-18-0373. Epub 2018 Apr 3. PMID: 29615459; PMCID: PMC6030480.
4) Ferrari SM, Elia G, Ragusa F, Ruffilli I, La Motta C, Paparo SR, Patrizio A, Vita R, Benvenga S, Materazzi G, Fallahi P, Antonelli A. Novel treatments for anaplastic thyroid carcinoma. *Gland Surg.* 2020 Jan;9(Suppl 1):S28-S42. doi: 10.21037/gs.2019.10.18. PMID: 32055496; PMCID: PMC6995904.